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13. ABSTRACT (Maximum 200 words) This project examined the efficacy of the heat-labile enterotoxin from <i>Escherichia coli</i> (LT) as an adjuvant for an inactivated HSV-2 vaccine. Using 5-week-old BAB/c mice, three oral doses of inactivated HSV-2/LT (25 µg/25 µg) vaccine given at weekly intervals were ineffective in protecting mice from infection. Administering the second booster of vaccine intravaginally (IVAG) or reducing the dose of LT (10 µg) did not increase the efficacy of the vaccine. This lack of response to the vaccine was not age-related as 8-week-old mice gave similar results, even when the primary vaccination was given IVAG. Finally, we tested different doses of inactivated HSV-2 in a protocol which included an oral vaccination on day 0 and 7 with 10 µg LT and either 1 µg, 5 µg, or 10 µg inactivated HSV-2. This was followed by an IVAG booster on day 14. Our results showed significant protection ($P < 0.05$) in the 10 µg HSV-2/LT treated group as evidenced by increased survival and decreased vaginal HSV-2 titers.				
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FINAL REPORT

GRANT #: N00014-93-1-0747

PRINCIPAL INVESTIGATOR: Dr. Angelo J. Pinto

INSTITUTION: The Medical College of Pennsylvania

GRANT TITLE: Efficacy of the Heat-Labile Enterotoxin From *Escherichia coli* as an Adjuvant for an HSV-2 Inactivated Oral Vaccine

AWARD PERIOD: 1 June 1993 - 31 March 1996

OBJECTIVES: The specific objectives of this study were to: (i) establish whether the heat-labile enterotoxin from *Escherichia coli* (LT) is an effective adjuvant for an orally administered killed HSV-2 vaccine, (ii) determine the ability of the vaccine to induce local and systemic humoral immunity, and (iii) examine the effect of this vaccine on HSV-2 pathogenesis.

APPROACH: To determine an effective vaccination protocol, various amounts of UV inactivated HSV-2 and LT are administered orally. Mice are then challenged intravaginally (IVAG) with live HSV-2 and monitored for illness, mortality, and HSV-2 titers in vaginal secretions. Once an effective vaccination protocol is established, vaccinated mice are monitored for HSV-2 replication in various organs, and local and humoral antibody is measured by ELISA and virus neutralization.

ACCOMPLISHMENTS: We have tested different vaccination protocols for their effectiveness in protecting mice against IVAG challenge with HSV-2. In the first year of this study we noted that vaccination with the oral HSV-2/LT vaccine increased susceptibility to infection. In the second year we tested the vaccine in different schedules and did studies that included IVAG immunization.

In the first vaccination protocol tested, there were four groups. The vaccinated group (HSV-2/LT) received 25 µg UV-inactivated HSV-2 orally with 25 µg LT on days 0, 7 and 14. The other three groups were the virus control group (received inactivated HSV-2 alone), the LT control group (received LT alone) and the vehicle control (received PBS). On day 21 all mice were challenged IVAG with 5×10^4 PFU HSV-2. The results of this experiment showed that the vaccinated mice (HSV-2/LT) and the virus and LT controls were equally susceptible to IVAG challenge with HSV-2, as shown by the severity of vaginitis, levels of HSV-2 found in vaginal secretions and systemic spread and mortality.

To assess whether the efficacy of the vaccine could be improved, we next tested protocols that included IVAG immunization and a lower dose of LT (10 µg). In one experiment, mice were treated as above except that the day 14 treatments were given IVAG. Mice receiving the HSV-2/LT vaccine or LT alone were more susceptible to infection than the corresponding virus control group as judged by grade of vaginitis and local vaginal replication of HSV-2. This increased susceptibility occurred when LT was used at either a 25 or 10 µg dose.

One problem we encountered was age-related resistance of BALB/c mice to IVAG infection with HSV-2. Thus, in the above experiments mice were 5 weeks old at the time of the primary vaccination. To test whether the poor response to the vaccine was age related, we did experiments using 8 week old mice treated with Depo-Provera (2.5 mg one week before and the day of infection). Depo-Provera renders older mice uniformly susceptible to IVAG infection with HSV-2. In one experiment we vaccinated mice orally with 25 µg inactivated HSV-2 and 10 µg LT on days 0, 7 and 14, followed by an IVAG booster on day 21. The results showed no protection from infection. As in previous experiments, the HSV-2/LT and LT treated mice showed more severe vaginitis but the effect was not as dramatic as previously noted. Next we tried a primary IVAG immunization on day 0 followed by secondary oral treatments on days 7 and 14. When the Depo-Provera treated mice were challenged with HSV-2 on day 28 all mice developed severe vaginitis and 100% mortality by day 7. Similarly, when three IVAG immunizations were given on days 0, 14 and 28 all groups developed severe vaginitis and mortality by day 7.

In our last experiments we tested different amounts of HSV-2 antigen. Up to this point all vaccinations had been done with a 25 µg dose of inactivated HSV-2. The vaccination protocol included oral vaccination on day 0 and 7 with 10 µg LT and either 1 µg, 5 µg, or 10 µg inactivated HSV-2. This was followed by an IVAG booster on day 14. Our results showed significant protection ($P < 0.05$) in the 10 µg HSV-2 treated group as evidenced by increased survival (90% in the HSV-2/LT treated group vs. 10% in the HSV-2 treated group) and decreased vaginal HSV-2 titers.

CONCLUSIONS: Our overall conclusion is that LT is an effective adjuvant for use in an inactivated HSV-2 vaccine. The effectiveness of LT is dependent on the dose of HSV-2 used in the vaccine. High doses of inactivated HSV-2 given with LT render mice more susceptible to infection following IVAG challenge with live HSV-2. In these studies the optimal dose of inactivated HSV-2 to use with LT was 10 µg.

SIGNIFICANCE: Our results have provided important information on the design of an effective vaccination protocol using LT as an adjuvant to increase the efficacy of a killed HSV-2 vaccine.

PATENT INFORMATION: None

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PUBLICATIONS AND ABSTRACTS (for total period of grant)

1. Pinto, A.J. and Tull, T. (1996). Use of the heat-labile enterotoxin from *Escherichia coli* to increase the efficacy of an inactivated HSV-2 vaccine. (In preparation).